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EFFECT OF SOME VEGETABLE BLENDS ON IRON BIOAVAILABILITY

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ABSTRACT

Spinach with one or more of some vegetables (chard, coriander lea and chickpea) rich in iron were used to prepare traditional household diets or blends. So, this work was designed to study the chemical composition of these vegetables and their content from some minerals (iron, calcium and phosphorus) and ascorbic acid to findout a natural sources of iron for use as food fortification. The effects of prepared diets or blends on iron bioavailability, final body weight, body weight gain, food intake, food efficiency ratio and some organs weight of rats were studied. The results revealed that:

Spinach contains the highest significant values of protein, ash and crude fiber followed by chard, coriander lea and chickpea, respectively. Meanwhile, chickpea had the highest values of carbohydrate and fat. In relation to iron content, coriander lea had the highest percent (43.905 mg/100 g) followed by spinach (37.783 mg/100 g) than chard (30.211 mg/100 g). Calcium content showed the same trend of iron. Chard and spinach had the highest content of phosphorus. Regarding to ascorbic acid as an important component in the vegetables, coriander lea recorded the highest amount of ascorbic acid (590.5 mg/100 g) followed by chard and spinach (425.6 and 370.2 mg/100 g), respectively. Chickpea gave the lowest percentage of all determined minerals and ascorbic acid.

In relation to final body weight, body weight gain, food intake and food efficacy ratio, the rats fed on the diet fortified with spinach, coriander lea and chickpea (diet F) were recorded the highest values of all previous parameters comparing with anaemic control, normal control or diet fortified with ferrous sulphate (diet A). Other prepared fortified diets revealed improvement of all these parameters when compared with anemic control or normal control. Concerning some organs weight and organs ratio, the results showed that, anemic rats which fed on iron free basal diet throughout the experimental period gave the lowest mean values of all organs weighed and organs ratio except the heart, which gave the highest weight and ratio. This may be due to cardiomegaly in consequence of iron deficiency anemia. In this relation, all prepared fortified diets improved organs weight and ratio.

The obtained results indicate that, there was positive effect on all blood parameters. An increase in haemoglobin, haemotocrite and serum iron was observed in the experimental animals which suffered from iron deficiency anemia due to the consumption of diets containing vegetable blends. The most positive effect was noticed with rats fed on the diet containing spinach, coriander and chickpea (diet F). All tested diets gave a normal concentration of haemoglobin (12-18 g/100 ml), normal percentage of haemotocrite (39-54%) and normal concentration of serum iron (70-180 µg/dl), this mean that, these diets can be used to protect from iron deficiency anemia.

INTRODUCTION

Iron deficiency anemia is currently the most frequent nutritional disorder worldwide, with different countries being affected to a different extent. Iron deficiency is estimated to affect more than 20% of the world population (INACG, WHO, UNICEF, 1998, Martinez-Navarrete *et al.,* 2002; de Almeida *et al.,* 2002 and Sawnson, 2003). Iron is a major component of haemoglobin that carries oxygen to all parts of the body. Iron has also a critical role within cells assisting in oxygen utilization, enzymatic system, especially for neutral development and overall cell function everywhere in the body (Alton, 2005 and Bersamin, 2004).

In general, individuals at greatest risk of iron deficiency anemia are those with increased iron needs. These groups include older infants and toddlers, teenage girls, women of childbearing especially those with high menstrual losses, pregnant women and women who breast feed (WHO, 1996 and FNBIM, 2001). Prevalence of anemia in any society, further consequences become more evident including changes in behaviour and intellectual performance, reduced resistance to infection, increased susceptibility to lead poisoning, loss of appetite, tachycardia and cardiomegaly (Bersamin, 2004).

In Egypt, anemia prevalence among preschool children is 40% (UNICEF, 1991 and Abd Rabou, 1994). The information published in Egypt by National Nutrition Surveys (1978-1980) revealed that iron deficiency anemia in Egypt is principal due to malnutrition especially insufficient available iron where, 14% of pregnant women and women who breast feed also 52% of children used to get less than 75% of Recommended Daily Allowances (RDA) of iron. So, food fortified with iron is generally considered the most effective way to increase iron intake and can be achieved by fortifying widely consumed foodstuffs which provide iron to all segments of the population.

Dietary iron is found in two forms, heme and non-heme iron. Heme iron, found in red meat, fish and poultry, is readily absorbed regardless of the other components in a meal. By contrast, non-heme iron found in both plant and animal sources, is absorbed to a much smaller degree, and is frequently affected by other food constituents. Non-heme iron accounts for more than 85% of the iron in the diet. Good sources of it include dried apricots, oatmeal, spinach, coriander lea, pine nuts, beans and iron fortified foods breads and cereals. In order to maximize iron uptake, foods high in non-heme iron should be eaten at the same time as those are a good source of vitamin C (Taylor *et al.,* 1986; Hallberg *et al.,* 1989; NRC, 1989; Hallberg *et al.,* 1993; Allen & Ahluwalia, 1997; Heath & Fairweather-Tait 2002; Swanson, 2003 and Bersamin, 2004).

The success of iron fortification depends on the bioavailability of the iron compound employed, the inhibitory and enhancing effects on absorption of the meals in which it is eaten, the level of addition and the consumption patterns of the medium to which it is added. Iron compounds commonly used and their relative bioavailability values with respect to ferrous sulphate (reduced iron). Fortification is technically more difficult for iron than for many other nutrients. Compounds with the highest bioavailability are soluble in water or dilute acid and tend to react with other food elements to cause off-flavours, changes in colour and fat oxidation. Another problem concerning the choice of the iron salt is that about 8% of patient depend on iron therapy have side effects, ranging from nausea and vomiting to diarrhea and sever stomach cramp (Heath & Fairweather, 2002; Lynch, 2005 and Yang & Lawless, 2006). Recently, Lund *et al.* (1999) found that oral ferrous sulphate supplements increase the free radical generating capacity of feces from healthy volunteers. In contrast, there are insufficient studies about the potential use of the natural sources of dietary in fortification of meals.

Spinach, chard and coriander lea are three of vegetables with the highest amount of chlorophyll, fat-soluble substances that stimulate haemoglobin and red blood cells production. Chlorophyll is known to have a chemical formula remarkably similar to that of haemoglobin and it has been said that the ingestion of chlorophyll will raise the haemoglobin in blood without increasing the formed elements. These vegetables are good sources for many vitamins e.g. vitamin C, E, A, folate …. and minerals Fe, Mn, Ca, Na, P and crude fiber. While spinach is known for its high iron content, recent studies have shown that the iron contained in spinach is not easy for the body to assimilate and only a small amount is used, chard and coriander lea are excellent sources of iron (Ensminger and Esminger, 1986; Fortin, 1996; USDA, 1998, Maybaum, 2002 and Morris *et al.,* 2006). Chickpea is know as a good source of in expensive dietary protein and other nutrients especially in the developing and underdeveloped countries. Using of chickpea in diets or in some snack products can reduce the levels prevalence of anemia among the population of young children and cover a high percentage of RAD of iron (Cardoso and Areas, 2001 and Cardoso *et al.,* 2001).

The preferred approach to increase iron intake of most severely groups of iron deficiency would be targeted to fortification of some foods that is used selectively in this age groups. Some vegetables can be used as it is and another can be used in blends (Hallberg, 1996).

There are many blends of spinach are made as a traditional diets e.g. spinach with chard, spinach with coriander lea, spinach with chickpea and spinach with coriander lea and chickpea. The objective of this study was to find out the effect of some household blends of some vegetables rich in iron such as spinach, chard and coriander as well as chickpea on some parameters of anemia, some organs weight, in addition to body weight gain and food efficiency ratio and to compared these blends with the common commercial fortifier ferrous sulphate.

MATERIALS AND METHODS

Materials:

Spinach (*Spinacia oleraceae* L.), chard (*Beta vulgaris* var. *cicla*) and coriander lea (*Coriandrum sativum*) were obtained from local market, while chickpea (*Cicer arietium*) variety Giza 2 was obtained from Agriculture Research Center (ARC) Giza, Egypt.

Ferrous sulphate ($Fe\ SO_4.7H_2O$), different mixtures of minerals (salt mixture and iron free salt mixture) were purchased from Sigma Chem. Co.

Animals:

Weaning female albino rats of body weight (42-50 g) were obtained from Faculty of Science, Tanta University.

Methods:

Preparation of raw materials:

Fresh vegetables were cleaned from any foreign materials, washed and cooked on direct heat until they cooked and appear to be free from moisture, then dried in an electric oven under vacuum at 55ºC $\pm$ 5ºC. After drying, vegetables were ground up to pass through 100 mesh screen sieve, then kept in polyethylene bags and stored at -18ºC $\pm$ 2ºC until analyzed or used. Chickpea was cleaned and separated from foreign matters, then grounded and kept as mentioned previously.

Preparation of experimental diets:

The composition of basal diet as recommended by Kim and Shin (1998) is illustrated in Table (A). Other experimental diets used in this work which were fortified with ferrous sulphate and blends of spinach are presented in the same Table.

Table (A): Composition of experimental diets (g/kg diet) as reported by Kim and Shin (1998).

Constituents	Basal diet	Basal depletion diet	Diet (A)	Diet (B)	Diet (C)	Diet (D)	Diet (E)	Diet (F)
Casein	200	200	200	200	200	200	200	200
Wheat starch	698	698	698	398	398	398	398	398
Corn oil	50	50	50	50	50	50	50	50
Mineral mixtures*	35	-	-	-	-	-	-	-
Vitmains mixtures	10	10	10	10	10	10	10	10
DL-Methionine	3	3	3	3	3	3	3	3
Choline bitartarate	2	2	2	2	2	2	2	2
Cholesterol	2	2	2	2	2	2	2	2
Mineral mixtures iron free**	-	35	35	35	35	35	35	35
Ferrous sulphate	-	-	0.3	-	-	-	-	-
Spinach	-	-	-	300	210	210	210	210
Coriander lea	-	-	-	-	90	-	-	45
Chard	-	-	-	-	-	90	-	
Chickpea	-	-	-	-	-	-	90	45

*** Mineral mixtures consisted of (g/kg diet): calcium carbonate, 3.1; potassium citrate, 13.1; dicalcium phosphate, 5.13; magnesium sulphate, 5.1; sodium chloride, 7.2; ferric citrate (16.7% Fe), 1.2; potassium iodide, 0.034; manganese sulphate, 0.12; zinc chloride, 0.012 and cupric sulphate 0.004**

**** Mineral mixtures iron free consisted of the previous components without iron salt.**

Chemical analysis:

Proximate chemical composition including moisture, crude protein, ether extract, ash and crude fiber of raw materials were determined as described in the A.O.A.C. (1990). Total carbohydrate contents was calculated by difference. Iron and calcium were determined using Atomic absorption spectrophometer (Perkins-Elmer Instrument, Model, 2380) as described by A.O.A.C. (1990). Phosphorus content was estimated photometrically as described by Chapman and Pratt (1978). Ascorbic acid was determined using the 2, 6 dichlorophenol indophenol tirtration method as described by A.O.A.C. (1990), results were expressed as mg ascorbic acid/100 g sample.

Bioavailability of iron:

Depletion repletion method for determining the bioavailability of spinach blends was carried out according to Ranhotra *et al.* (1983).

Animals:

Forty weaning female albino rats of body weight 42-50 g were housed individually in a wire bottom cages under normal healthy laboratory conditions. Temperature and humidity were ranged from 20-25^{o}C and 60-65%, respectively. Diet and water were provided adlibitum.

Depletion and repletion assay:

Thirty five rats were fed on the free iron depletion diet (basal depletion diet) for 40 days until the blood haemoglobin level dropped to about 9.5-10.5 g/100 ml. Another five rats were used as control group without iron depletion. Rats were fed on depletion diet for forty days, then blood samples were collected from the tested rats for biochemical analysis (haemoglobin, haemotocrite and serum iron).

The depleted rats (anemic rats) were divided randomly into seven groups of five rats. One group kept as anemic control which fed on basal diet with iron free salt mixture (basal depletion diet) until the end of experimental period. The other six groups were fed on the basal diet fortified with ferrous sulphate and blends of vegetables for five weeks as shown in Table (B). Rats were weighted weekly and food intake was recorded daily. At the end of the experiment, rats were sacrificed. Blood samples were collected and the organs were weighed.

Table (B): The groups of albino rats and types of diet used in the experiment.

Rat groups	Types of diet
Normal control	Basal diet with iron, this group had no iron depletion.
Anemic control	Basal diet iron free (basal depletion diet).
Group A	Basal diet iron free fortified with 300 mg ferrous sulphate/kg diet.
Group B	Basal diet iron free fortified with 300 g spinach/kg diet (diet B).
Group C	Basal diet iron free fortified with 210 g spinach + 90 g coriander/kg diet (diet C)
Group D	Basal diet iron free fortified with 210 g spinach + 90 g chard/kg diet (diet D).
Group E	Basal diet iron free fortified with 210 g spinach + 90 g chickpea/kg diet (diet E).
Group F	Basal diet iron free fortified with 210 g spinach + 45 g coriander + 45 chickpea/kg diet (diet F).

Biochemical analysis:

Five ml intravenous blood were withdrawn from each rat, one ml was put in a tube containing EDTA (ethylene diamine tetra acetic acid) as anticoagulant and shacked well and used for haemoglobin and haematocrite determination. The rest of the sample (4 ml) was transferred to sterilized dry centrifuge tubes, left to cool and the serum was separated after centrifuged for 10 min. at 70000 rpm, the serum was kept frozen at -20ºC in dry clean plastic tubes for determination of serum iron. The blood haemoglobin concentration, haematocrite value and serum iron were measured as recommended by Dacie and Lewis (1984).

Statistical analysis:

The data were statistically analyzed using the analysis of variance and the means were further tested using the least significant difference test as outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical composition of raw materials:

Results in Table (1) show the chemical composition of spinach, coriander lea, chard and chickpea. The results indicate that spinach had a significantly higher crude protein content (31.954%) followed by chard (26.523%) and coriander lea (25.885%), while chickpea has the least one. These results are in full agreement with the results of Maybaum (2002) who reported that spinach contains more protein than most vegetables The previous results also are in accordance with these found by Gordan & Chao (1984) and Ribeiro & Melo (1990). Data in Table (1) also indicate that chickpea had a significantly higher fat content (5.74%) and the other vegetables had a fair percentage of fat compared with chickpea, these percentages were 5.153, 4.457 and 3.725% for coriander lea, spinach and chard, respectively. In relation to ash and crude fiber content, spinach and chard had the highest amount of ash (21.065 and 22.111%) and crude fiber (23.122 and 21.095%), respectively, and chickpea had the lowest values (3.57 and 5.27%). In contrast, the highest carbohydrate content was recorded with chickpea (67.632%). The aforementioned results coincide with those obtained by (Ribeiro & Melo, 1990, USDA, 1998 and Zein, 2005).

Table (1): Chemical composition of spinach, chard, coriander lea and chickpea (g/100 g on dry weight basis).

Raw materials	Moisture	Crude protein	Crude fat	Ash	Total carbohydrates	Crude fiber
Spinach	90.80 a	31.954 a	4.457 c	21.065 a	42.524 d	23.122 a
Chard	91.65 a	26.523 b	3.725 d	22.111 a	47.641 c	21.095 b
Coriander lea	91.75 a	25.885 b	5.153 b	15.000 b	53.962 b	12.811 c
Chickpea	10.58 b	23.058 c	5.740 a	3.570 c	67.632 a	5.270 d

Each value was an average of three determinations.
Values followed by the same letter are not significantly different at P $\leq$ 0.05.

Data presented in Table (2) showed some minerals and ascorbic acid content of spinach, chard, coriander lea and chickpea. The results reveled that, all materials used are good sources for iron, calcium, phosphorus and ascorbic acid, except chickpea. The highest amounts of iron, calcium and ascorbic acid were recorded with coriander lea, the amounts were 43.905, 1125.0 and 590.50 mg/100 g on dry weight basis, respectively. Also, spinach and chard contained high amounts of iron calcium, phosphorus and ascorbic acid more than that found in chickpea, so, chickpea is a poor source of iron and ascorbic acid. These results are closely related to those reported by USDA (1998) and CGC (2004). Also, GMF (2007) published that, chard is an excellent source of iron and vitamin C, as well as very good source of calcium and phosphorus.

Table (2): Iron, calcium, phosphorus and ascorbic acid contents of spinach, chard, coriander lea and chickpea (mg/100 g on dry weight basis).

Raw materials	Iron	Calcium	Phosphorus	Ascorbic acid
Spinach	37.783	989.330	565.00	370.20
Chard	30.211	715.200	597.20	425.60
Coriander lea	43.905	1125.00	485.50	590.50
Chickpea	8.248	135.410	330.00	4.60

Effect of feeding anemic rats on vegetable blends on final body weight, body weight gains, food intake and food efficiency ratio (FER):

Results illustrated in Table (3) revealed that, no significant differences were observed in initial body weight between all groups after depletion period, the initial body weight ranged between 71.82 and 74.13 g. The same results showed clearly that the rats fed on basal depletion diet fortified by spinach, coriander lea and chickpea (group F) had significantly the highest final body weight (105.31 g), body weight gain (31.51 g) and body weight gain percentage (42.7%) with an amount of food intake equal to 205.24 g and food efficiency ratio (15.35%).

Table (3): Effect of feeding anemic rats on prepared diets on final body weight, body weight gains, food intake and food efficiency ratio (FER).

Dietary group	Initial body weight (g)	Final body weight (g)	Body weight gain		Food intake (g)	Food efficiency ratio (FER)
			g	%*		
Normal control	73.50 a	96.85 bc	23.35 bc	31.77 cd	168.80 d	13.83 d
Anemic control	72.80 a	94.35 c	21.55 c	29.60 d	155.69 e	13.84 d
Group A	72.65 a	98.22 b	25.57 bc	35.20 cd	179.65 c	14.23 c
Group B	73.91 a	99.02 b	25.11 bc	33.97 cd	192.82 b	13.02 f
Group C	73.10 a	98.15 b	25.05 bc	34.27 cd	190.30 b	13.16 e
Group D	74.13 a	103.65 a	29.52 a	39.82 ab	195.95 b	15.07 b
Group E	71.82 a	99.00 b	27.18 b	37.84 bc	196.25 b	13.85 d
Group F	73.80 a	105.31 a	31.51 a	42.70 a	205.24 a	15.35 d

Each value is an average of five determinations

Means values followed by the same letter in column are not significantly different at P $\leq$ 0.05

*** Body weight gain (%) = Final body weight-initial body weight/initial body weight x 100**

**** Food efficiency ratio = Body weight gain/food intake x 100**

This group was followed by group (D) which fed on basal depletion diet fortified with spinach and chard (diet D). This may be attributed to the differences of chickpea and chard protein and other chemical composition. On the contrary, anemic control rats which fed on basal depletion diet (iron free) had significantly the lowest values of final body weight (94.35 g), body weight gain (21.55 g) and food intake (155.69 g) when comparing with normal control, group (A) which fed on basal depletion diet fortified with ferrous sulphate (diet A) and other groups. These results are in agreement with those found by Epollot and Van (1997) who reported that, the body weight gain of the experimental rats depends on the content of protein and fat of their diets, while the iron content had no effect.

Effect of feeding anemic rats on vegetable blends on organs weight:

The organs (liver, spleen, heart and kidney) were weighed and the results are recorded in Table (4). The ratio between organ weight and final body weight was calculated. The obtained data indicated that, the rats of group (F) recorded significantly highest values and percentages of liver and kidney weight, while group (C) and group of anemic control gave the highest significant percentages of spleen and heart, respectively. On the other side, anemic control rats which fed on basal depletion diet without any fortification had significantly the lowest values and percentages of liver, spleen and kidney. The obtained results also indicated that an improvement could be observed for all groups fed on the tested vegetables blends and the diet fortified with ferrous sulphate. These results are in agreement with those of Zein (2005), who found that, fortification of pie with cooked spinach or ferrous sulphate led to an improvement of organ weight and ratio. Anemic control rats had the highest weight and percent of heart, this may be due to cardiomegaly in consequence of iron deficiency anemia (Bersamin, 2004).

Table (4): Effect of feeding anemic rats on prepared diets on organs weight.

Dietary group	Final body weight	Liver		Spleen		Heart		Kidney	
		g	R.O.W. %	g	R.O.W.%	g	R.O.W. %	g	R.O.W. %
Normal control	96.85 b	4.20 bc	4.337 b	0.45 c	0.465 bc	0.46 b	0.475 b	0.85 d	0.878 c
Anemic control	94.35 c	3.30 d	3.498 c	0.38 d	0.403 d	0.49 a	0.519 a	0.73 e	0.774 d
Group A	98.22 b	4.20 bc	4.276 b	0.45 d	0.458 c	0.45 b	0.458 b	0.92 c	0.937 bc
Group B	99.02 b	4.24 bc	4.282 b	0.46 c	0.464 bc	0.42 c	0.424 c	0.95 c	0.959 b
Group C	98.15 b	3.95 c	4.024 b	0.53 ab	0.540 a	0.38 d	0.387 d	0.95 c	0.968 b
Group D	103.65 a	4.97 a	4.795 a	0.53 ab	0.511 abc	0.48 a	0.463 b	1.00 ab	0.965 b
Group E	99.0 b	4.33 b	4.374 b	0.49 bc	0.495 abc	0.48 a	0.485 b	1.02 a	1.030 a
Group F	105.31 a	5.11 a	4.852 a	0.55 a	0.522 ab	0.49 a	0.465 b	1.06 a	1.006 a

Each value is an average of five determinations
Values followed by the same letter in column are not significantly different at $P \leq 0.05$
Relative organ weight (R.O.W.) = Organ weight/final body weight x 100

Effect of feeding anemic rats on vegetable blends on haemoglobin, haematocrite and serum iron:

The mean values of haemoglobin (g/100 ml) in blood of rats fed on all vegetable blends, basal diet fortified with ferrous sulphate and basal depletion diet are recorded in Table (5). From these results, it could be noticed that, haemoglobin values decreased in all groups after depletion period except normal control group because of the presence of iron in basal diet. After repletion period, haemoglobin values increased in all groups except the anemic control which decreased in haemoglobin value due to the absence of iron in basal depletion diet. At the end of experimental period, group rats (A) which fed on depletion basal diet fortified with ferrous sulphate (0.03%) recorded blood haemoglobin (13.85 g/100 ml), while group (F) had significantly the highest value of haemoglobin (15.75 g/100 ml), followed by group (C) and group (E) (15.0 g/100 ml). This may be attributed to presence of coriander lea and chickpea; coriander lea or chickpea in diet groups (F), (C) and (E), respectively. Coriander lea had the highest amount of iron and vitamin C (Table 2). Ascorbic acid increase the absorption of non heme iron that present in spinach, coriander lea and chard at high levels. The results of Hurrel (1984); Fleshood & Hallberg (1998); Martinez *et al.* (2004) and Zein (2005) coincide our results,. They reported that adding of ascorbic acid to tested meal led to increase the absorption of non heme iron because it acts as acidifying agent that enhance iron availability through the reduction of the less available ferric to more available ferrous forms. Also the enhancing effect of ascorbic acid by maintaining the solubility of non heme iron when the food enters the alkaline environment of the small intestine, has long been recognized as reducing the influence of the inhibitory ligands that bind iron in the more alkaline pH of the duodenum. El-Said (2001) and Zein (2005) indicated that pies made from wheat flour and fortified with cooked spinach or ferrous sulphate improved blood haemoglobin and iron deficiency in the anemic rats. Also, Al-Awady (1983) and Abd El-Lateef (2002) published that, legumes are rich in folacin, blood haemoglobin synthesis need to folacin for red blood cell.

Table (5): Effect of feeding anemic rats on prepared diets on haemoglobin, haematocrite and serum iron levels after depletion and repletion periods.

Dietary group	Haemoglobin		Haematocrite		Serum iron	
	AD	AR	AD	AR	AD	AR
Normal control*	13.82 a	14.35 c	45.33 a	43.65 ab	1635.49 a	134.15 c
Anemic control **	10.22 b	9.20 e	28.86 d	31.25 c	82.24 c	82.45 d
Group A	10.35 b	13.85 c	32.00 c	41.25 b	82.50 bc	154.20 b
Group B	10.53 b	12.35 d	33.50 bc	41.70 ab	83.45 bc	152.30 b
Group C	10.46 b	15.00 b	33.00 bc	44.00 ab	85.33 b	165.35 a
Group D	10.40 b	13.75 c	33.00 bc	43.33 ab	84.40 bc	163.55 a
Group E	10.00 b	15.00 b	32.50 bc	42.25 ab	83.45 bc	155.00 b
Group F	10.00 b	15.75 a	33.80 b	44.50 a	85.00 bc	165.00 a

Each value is an average of five determinations
Values followed by the same letter in column are not significantly different at P $\leq$ 0.05
AD after depletion period AR after repletion period
*** No iron depletion for the normal control**
**** No iron repletion for the anemic control.**
Normal value of haemoglobin: 12-18 g/100 ml (Rolfes, 1991)
Normal value of haemotocrite = 39-54% (Rolfes, 1991)
Normal value of serum iron = 70-180 µg/dl (Yip *et al.,* 1995).

In relation to haemotocrite, data in Table (5) shows that, haematocrite level of normal control rats was 45.33%, while the other groups fed with basal depletion diet (free from iron) throughout depletion period showed decrease. After repletion period and fortification of diets with ferrous sulphate or vegetable blends, significant differences were observed among all groups. Meanwhile, anemic control group fed on basal diet iron free throughout depletion and repletion periods showed significantly the lowest levels of haemotocrite (28.86 and 31.25%), respectively. In this respect group (F) which fed on diet fortified with spinach, coriander lea and chickpea (diet F) recorded significantly the highest level of blood haemotocrite. In the same trend, other groups recorded significant differences in blood haemotocrite comparing with anemic control, but had no significant difference comparing with normal control. These results are in good agreement with the results of Fbranklin and Rudman (1999), who indicated that, diets contain amounts of iron 8-12 mg/100 g and factors affecting iron bioavailability (ascorbic acid) led to increase haemotocrite concentration by 89-100% throughout 28 days of feeding.

In regard to serum iron, the mean values of rats fed on control diet or other tested materials are outlined in Table (5). It is apparent from the data that, anemic control had significantly the lowest mean value after depletion and repletion periods because no fortification by iron was carried out after depletion period. All vegetable diets and ferrous sulphate had a more effect on serum iron comparing with normal control or anemic control, but the highest values of serum iron were observed with group (C). This may be attributed to that diet (C) had the highest amount of iron (calculation regardless of the group of ferrous sulphate group (A). Group (F) and (D) had no significant differences comparing with group (C). The results of serum iron in Table (5) also revealed that, all mean values of all groups, normal control and anemic control, after depletion or repletion period not decreased or increased than the range (70-180 µg/dl) limited by Yip *et al.* (1995). Janet *et al.* (1990) and Hurrel (1997) reported that vitamin C improved the level of serum iron and increase the absorption of all fortification iron compounds to a similar extent until 10 folds. Cardoso and Areas (2001) and Cardoso *et al.* (2001) decided that snack contained chickpea, corn and bovine lung flour had a high quality protein content and provided 30-40% of iron RDA in 30 g pack and could be useful in nutritional programs against anemia and malnutrition.

As a conclusion, form the previous aforementioned results it should be noted that, all prepared diets or vegetable blends had high amounts of iron and vitamin C. Although fortification of diet with ferrous sulphate is considered way to increase iron intake and improve blood parameters. It well known that ferrous sulphate and other iron salts had many problems and side effects. So, we prefer to use the natural sources of dietary iron in food fortification and advise the consumers to use green vegetables rich in iron and vitamin C to protect themselves from iron deficiency or iron deficiency anemia.

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تأثير بعض خلطات الخضر على الاتاحة الحيوية للحديد

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تستخدم السبانخ مع واحد أو أكثر من الخضروات (السلق - الكسبرة الخضراء - الحمص) فى إعداد وجبات أو خلطات منزلية تقليدية. ولهذا فقد صمم هذا البحث لإيجاد مصادر طبيعية للحديد لاستخدامها فى تدعيم الأغذية ودراسة التركيب الكيماوى للخضروات السابقة ومحتواها من بعض العناصر مثل الحديد والكالسيوم والفوسفور وحمض الأسكوربيك بالإضافة إلى دراسة تأثير هذه الخلطات أو الوجبات على الإتاحة الحيوية للحديد ، وزن الجسم النهائى والزيادة فى وزن الجسم وكمية وكفاءة الطعام المتناول ووزن بعض أعضاء فئران التجارب.

وأوضحت النتائج ما يلى:

احتوت السبانخ على أعلى قيمة من البروتين الخام والرماد والألياف الخام تلاها بعد ذلك وجاء بعدها السلق والكسبرة الخضراء والحمص على التوالى ، بينما احتوى الحمص على أكبر نسبة من الكربوهيدرات والدهون. وفيما يتعلق بكمية الحديد ، احتوت الكسبرة الخضراء على أعلى نسبة من الحديد (٤٣,٩٠٥مللجم/١٠٠ جرام) تبعتها السبانخ (٣٧,٧٨٣مللجم/١٠٠ جرام) والسلق (٣٠,٢١١مللجم/١٠٠ جرام) وكانت نسب الكالسيوم تسير فى نفس اتجاه محتوى الحديد ، واحتوى السلق والسبانخ على أكبر كمية من الفوسفور. وبخصوص حمض الأسكوربيك كأحد المكونات الهامة فى الخضروات ، سجلت الكسبرة الخضراء أعلى قيمة من حمض الاسكوربيك (٥٩٠,٥مللجم/١٠٠ جرام) ثم تبعها فى ذلك السلق والسبانخ (٤٢٥,٦ ، ٣٧٠,٢مللجم/١٠٠ جرام) على التوالى. فى حين أعطى الحمص أقل نسبة فى كل من المعادن التى تم تقديرها وكذلك حمض الأسكوربيك.

فيما يتعلق بوزن الجسم النهائى والزيادة فى وزن الجسم وكمية وكفاءة الطعام المأكول ، سجلت مجموعة الفئران التى تم تغذيتها على الوجبة الأساسية الغير محتوية على الحديد والمدعمة بالسبانخ والكسبرة والحمص (diet F) أعلى قيم فى القياسات المذكورة سابقا عند مقارنتها بالقيم المتحصل عليها من المجموعة الضابطة المصابة بالأنيميا أو المجموعة الضابطة السليمة أو المجموعة المغذاة على الوجبة المدعمة بكبريتات الحديدوز. كل الوجبات الأخرى أدت إلى تحسين كل القياسات السابقة مقارنة بالمجموعة الضابطة المصابة بالأنيميا أو المجموعة الضابطة العادية. وبخصوص وزن بعض الأعضاء ونسبة هذه الأعضاء أظهرت النتائج أن المجموعة الضابطة المصابة بالأنيميا والتى تم تغذيتها على الوجبة الخالية من الحديد خلال فترة التجربة قد أعطت أقل متوسط لقيم وزن ونسبة الأعضاء التى تم تقديرها باستثناء وزن القلب ونسبة وزن القلب إلى الجسم فقد أعطت هذه المجموعة أعلى وزن للقلب وأعلى نسبة لوزن القلب إلى الجسم والتى من المعتقد أنها نتيجة لتضخم القلب الناشئ عن أنيميا نقص الحديد.

أشارت النتائج المتحصل عليها أن هناك تأثير إيجابى على كل قياسات الدم ، ولوحظ زيادة فى تركيز الهيموجلوبين وسيرم الدم وزيادة نسبة الهيماتوكريت فى حيوانات التجارب والتى كانت تعانى من أنيميا نقص الحديد وذلك نتيجة للتغذية علوجبات تحتوى على خليط الخضر. وقد لوحظ أن أكبر تأثير إيجابى كان مع مجموعة الفئران التى تم تغذيتها على الوجبة (F) المحتوية على السبانخ والكسبرة والحمص. وأعطت كل الوجبات المختبرة تركيزات طبيعية من الهيموجلوبين (١٢-١٨جم/١٠٠ مل) ، ونسبة طبيعية من الهيماتوكريت (٣٩-٥٤%) وتركيزات طبيعية من حديد السيرم (٧٠-١٨٠ ميكروجرام/١٠٠ مل) ، وهذا يعنى أن هذه الوجبات يمكن استخدامها للحماية من أنيميا نقص الحديد.